Prevalence of Multidrug-Resistant *Enterococcus faecalis* in Hospital-Acquired Surgical Wound Infections and Bacteremia: Concomitant Analysis of Antimicrobial Resistance Genes

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ABSTRACT

BACKGROUND: The study aimed to assess the prevalence of *Enterococcus faecalis* infections among patients with hospital-acquired surgical wound sepsis and bacteremia in surgical wards and identify the antimicrobial susceptibility in these pathogens. Genetic role of erythromycin, vancomycin, and cephalosporin resistance in these pathogens was also examined.

METHODS: Two hundred samples were collected from surgical wound infections and 100 blood cultures from patients with suggested bacteremia to identify *E faecalis* by phenotypic and genotypic methods. Antimicrobial susceptibility to 12 antimicrobial agents was tested. The presence of resistance genes was examined by polymerase chain reaction (PCR) assay.

RESULTS: *E faecalis* was isolated with a frequency of 24/200 (12%) from surgical wound samples and 2/100 (2%) from blood cultures. All isolates were completely resistant to cefepime, ampicillin, and tetracycline, 96% of isolates were resistant to erythromycin, 53.8% to vancomycin, and 23.1% to linezolid. Multidrug resistance (MDR) was found in 100% of isolates. *ere(B)* and *erm(B)* genes were present in 20/25 (80%) and 17/25 (68%) of erythromycin-resistant isolates, respectively, 15 (60%) isolates carry both *ere(B)* and *erm(B)* genes. *Van A* gene was detected in 71.4% of vancomycin-resistant isolates. All isolates were negative for *mef(A/E), blaSHV*, and *blaTEM* genes.

CONCLUSION: MDR in all isolates (100%) and high-level resistance to gentamicin, erythromycin, and vancomycin were reported in *E Faecalis* isolates. In the studied isolates, erythromycin resistance mainly related to the presence of *ere(B)* and *erm(B)* genes and vancomycin resistance was mainly related to the presence of *vanA* gene.

KEYWORDS: *Enterococcus faecalis*, multidrug-resistant (MDR), *ere(B)* gene, *erm(B)* gene, *vanA* gene

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Introduction

Enterococci, which were initially considered to be harmless flora of the gastrointestinal tract, have emerged in the last two decades as a major cause of hospital-acquired infections (HAIs),[1] including surgical site infections, urinary tract infections, and bacteremia.[1-3] In the past, the source of infection by enterococci was mainly endogenous[4]; after that, the transmission of enterococci among hospitalized patients was reported.[5] *Enterococcus faecalis* is one of the most common isolated pathogens from all types of wounds,[6,7] and the third frequent isolated pathogen from surgical site infections.[8,9] They are also account to be an important cause of bacteremia all over the world.[10] Colonization of the hands of health care workers by *E faecalis* can be a source of infection by contact with surfaces, or medical equipment[11] due to its ability to survive on inanimate surfaces, as well as on the hands of hospital staff for long time.[12] Treatment of *E faecalis* infections is so difficult because they have intrinsic and acquired resistance to many antimicrobials.[13] They have intrinsic resistance against a number of antimicrobials including, aminoglycosides and β-lactams due to carrying several resistance genes[14] as well as acquired resistance against several antibiotics like macrolides, vancomycin, cephalosporin, tetracycline, and fluoroquinolones, resulting from either DNA mutation or acquisition of new genes through gene transfer.[15] Most hospital strains are resistant to a wide range of antibiotics, including macrolide and vancomycin,[15-17] and also have been recognized as β-lactamases producers, causing resistance to penicillins and cephalosporins.[18] Few studies have focused on *E faecalis* isolated from surgical wound infections and bacteremia. In this regard, little is known about the prevalence of *E faecalis* isolated from surgical wound infections and bacteremia, their antimicrobial susceptibility, and the mechanisms of antibiotic resistance particularly in developing countries, which are investigated in the current study

Patients and Methods

Patient population

This is a cross-sectional study including 300 patients who developed clinical signs of surgical wound infection and bacteremia

Table 1. Primers sequences used for PCR assays.

GENES	PRIMER SEQUENCE	REFERENCE
ddl E Faecalis	F: ATCAAGTACAGTTAGTCT R: ACGATTCAAAGCTAACTG	Duka et al[21] Drahovska et al[22]
van A	F: GGGAAAACGACAATTGC R: GTACAATGCGGCCGTTA	Duka et al[21]
ere(B)	F: 59-AGA AAT GGA GGT TCA TAC TTA CCA-39 R: 59-CAT ATA ATC ATC ACC AAT GGCA-39	Portillo et al[23]
blaSHV	ATTTGTCGCTTCTTTACTCGC TTTATGGCGTTACCTTTGAC	Jemima and Verghese[24]
blaTEM	ATGAGTATTCAACATTTCCG CCAATGCTTAATCAGTGAGG	Tofteland et al[25]
erm(B)	F: GAAAAGGTACTCAACCAAATA R: GTAACGGTACTTAAATTGTTTAC	Sutcliffe et al[26]
mef(A/E)	F: AGTATCATTAATCACTAGTGC R: AGTATCATTAATCACTAGTGC	Sutcliffe et al[26]

at least 48 hours after hospital admission as identified by the Centers for Disease Control and Prevention National Healthcare Safety Network (CDC/NHSN),[19] from surgery departments at Minia University hospital (a teaching hospital provides care to adult and pediatric patients in 35 wards including 800 beds), between June 2017 and January 2018. Samples were collected as the following: 200 wound swaps from patients with clinical signs of septic wounds and 100 blood cultures from patients with suggested bacteremia. The study protocol was approved by the local institutional review board at the authors' affiliated institution (Registration number: MUH15329) and consents were obtained from all participants.

Bacterial isolation

Identification of the isolated *enterococci* to the genus level was performed by Gram staining, blackening of Bile Aesculin Azide Agar (Oxoid), culture on nutrient broth at 10°C, 45°C, and with 6.5% NaCl, then identification to the species level was performed by motility test, sugar fermentation tests (L-Arabinose, Mannitol, Sorbitol Glycerol D-Lyxose Mannitol, Galactose, and Hippurate), and arginine dihydrolase and pyruvate utilization test. Also identification of *E faecalis* strains confirmed by detection of *E Faecalis* gene using real-time polymerase chain reaction (PCR).

Antimicrobial susceptibility testing

Antimicrobial susceptibility of the isolates was determined by disk diffusion method for the following antibacterial agents; erythromycin (15 µg), gentamicin (120 µg), tetracycline (30 µg), ampicillin (10 µg), amoxicillin-clavulanic (30 µg), Cefepime (30 µg), vancomycin (30 µg), teicoplanin (30 µg), linezolid (30 µg), ciprofloxacin (5 µg), Imipenem (10 µg), and rifampin (5 µg) (Bioanalyse, Turkey). Muller-Hinton agar plates were inoculated with 0.5 McFarland standard suspension of the strains, antimicrobial disks were placed into plates and then were incubated at 37°C for 24 hours. Minimum inhibitory concentrations (MICs) of vancomycin and erythromycin were determined by the agar dilution method. Zone diameters were assessed according to the Clinical Laboratory Standard Institute guidelines.[20]

DNA extraction

DNA was extracted using genomic BYF DNA extraction Mini Kit (Intron Biotechnology, Korea) according to the manufacturer's instructions.

Identification of *E faecalis* Gene and Resistance Genes Using Real-Time PCR

The *ddl E faecalis* gene, *ere(B)* gene for erythromycin resistance, *van A* gene for vancomycin resistance, *blaSHV* and *blaTEM* genes for extended spectrum beta-lactamase (ESBL) production were amplified by PCR in all isolates. The primer sets used (Eurofins, Germany) for amplification of *ddl E faecalis*,[21,22] *van A*,[21] *ere(B)*,[23] *blaSHV*,[24] and *blaTEM*[25] genes are shown in Table 1. PCR was performed in 20 µL; 10 µL Hot Start Maxima SYBR green qPCR Master Mix (2X), 10 pmoles/µL forward primer and 10 pmoles/µL reverse primer (Macrogen, Korea), 0.05µL ROX solution, 200 ng of DNA and completed to 20 µL with nuclease-free water. PCR reactions were performed using real-time thermal cycler (Applied Biosystem 7500 fast), with a fluorescence detector. Each sample was tested in duplicate in the same Reverse Transcriptase PCR experiment. Standard curves and other data analysis were analyzed with Applied Biosystem Real-time Software.

Detection of erythromycin resistance genes, erm(B), and mef(A/E) by conventional PCR

PCR reactions were performed using thermal cycler (UNO II thermocycler, Biometra, Germany), 50 µL reaction: 25 µL DreamTaq Green PCR Master Mix (2X), 20 pmoles/µL for forward and reverse primer, 300 ng of DNA and completed to 50 µL with nuclease-free deionized water. Each gene was amplified using its specific primer[26] (Table 1) (Eurofins Genomic Co., Germany). Positive and negative controls from previous research were used.[27] PCR products were resolved on 2% agarose gel and visualized under a UV transilluminator (Biometra).

Statistical analysis

Categorical variables were analyzed using the chi-square test, using SPSS software (version 20). *P* values of $< .05$ were considered to be statistically significant.

Results

Characteristics of the study population

Out of 300 bacterial isolates, 26 (8.6%) *E faecalis* isolates were identified. Only one isolate per patient was detected. The majority of them (24/26) were isolated from patients with surgical wound infections. *E faecalis* was recovered with a frequency of 24/200 (12%), from surgical wound samples and 2/100 (2%) from blood cultures as shown in Table 2.

Antimicrobial susceptibility

Among the 26 tested *E faecalis* isolates, 100% were resistant to cefepime, ampicillin, and tetracycline, 25 (96%) to erythromycin, 22/26 (84.6%) to rifampin, 21 (80%) to gentamicin (120 µg), 18 (69.2.8%) to amoxicillin-clavulanic, 15 (61.5%) to ciprofloxacin, 14 (53.8%) to vancomycin, 6 (23.1%) to linezolid, 5 (19.2%) to teicoplanin, and 2 (7.6%) to imipenem. For vancomycin, MIC of all vancomycin-resistant isolates were ≥ 128 µg/mL. Multidrug resistance (MDR) was detected in all isolates (100%) as shown in Figure 1 and Table 2.

Detection of resistance genes

Out of 25 erythromycin-resistant isolates, 20 (80%) were found to be positive for *ere(B)* gene, 17 (68%) were found to be positive for *erm(B)* gene (Figure 2), all (26) *E faecalis* isolates were negative for *mef(A/E)* gene. Fifteen isolates carry both *ere B* and *erm(B)* genes, while 2 isolates carry *erm(B)* gene only and 5 isolates carry *ere(B)* gene only. *Van A* was detected in 10/14 (71.4%) of vancomycin-resistant isolates. Genes encoding–lactamases (*blaTEM* and *blaSHV*) were not detected in any of isolates (Table 2).

Discussion

Enterococci are frequently isolated from health care settings. They reported as the third most common hospital-acquired pathogen.[28] They are increasingly isolated from traumatic and surgical wounds[29] and from bacteremia.[10] In the present study, the prevalence of E. faecalis isolation was 8.6% among 300 Egyptian patients with hospital-acquired infections. The isolation rate was higher in surgical wound samples; 24/200 (12%) than blood samples (2%). Our findings were higher than other reports, where *E faecalis* was isolated from wound swabs with percentages of 6%[30] and 1.3%,[31] and were lower than others where the isolation rate from blood cultures was (4.6%).[32] Our study showed that all isolates were reported to be resistant to cefepime, ampicillin, tetracycline, and exhibited high resistance rates; (84.6%) to rifampin, and (69.2%) to amoxicillin-clavulanic, that are comparable to other reports.[27,33,34] In the present study, high-level resistance gentamicin (HLGR) (120 µg) rate was (80%), vancomycin resistance rate was (53.8%), and teicoplanin resistance rate was (19.2%). The frequency of HLGR and glycopeptide resistance in the current study were very high compared with those of previous studies from Egypt.[27,35,36] Therefore, our study reveals increasing rates of resistance to gentamicin and glycopeptide, which makes the reassessment of antibiotics regimens in Egypt is very important. Erythromycin resistance in the current study was (96%), which was comparable to some reports[37] and higher than others.[17,27,33] Linezolid resistance rate in the current study was (23.1%) which was higher than that of other reports[27,32] while (100%) sensitivity to linezolid was reported in several studies.[16,37] Regarding imipenem, resistance rate was (7.6%), which was lower than other reports[37] and higher than others,[38] so, linezolid, teicoplanin, and imipenem may be the alternative treatment for hospital-acquired infections caused by *E faecalis*.

Multidrug resistance (MDR) was detected in all isolates (100%), defined by resistance to three or more Antimicrobials from different antimicrobial families[39] indicating a big challenge in treating infections by *E faecalis* with empirical regimens in Egypt. This MDR rate is comparable to that of previous reports.[27,33] High level of resistance to these antibiotics is likely related to the wide use of these antibiotics for treatment of gram-positive infections in our locality.

Macrolides are still effective for treatment of important human infections.[40] Cross-resistance to macrolides is caused by mutations in *erm* genes encoding methylases and/or 23 S rRNA.[41] Increasing rate of mutations in *erm* genes and appearance of different resistance mechanisms among the clinical pathogens show a complexity of resistance to macrolides, so studying such mechanisms between the *enterococcus* isolates is still important.[41] Regarding identification of erythromycin resistance mechanisms in our isolates, we found 20/26 (76.9%) of all isolates and 20/25 (80%) of erythromycin resistant isolates were positive for *ere(B)* gene, 17/26 (65.4%) of the total *E faecalis* isolates and 17/25 (68%) of erythromycin resistant isolates were positive for *erm(B)* gene. However, all (26) *E faecalis* isolates were negative for *mef(A/E)* gene. Fifteen isolates carry both *ere(B)* and *erm(B)* genes. Our findings agreed with Bello Gonzalez et al,[42] who reported that 8/13

Table 2. Characterization of *E Faecalis* isolated from hospital-acquired surgical wound infection and bacteremia.

ISOLATE NO.	LOCATION OF PATIENT	SPECIMEN	ERYTHROMYCIN-RESISTANT GENES			BLA GENES	VANCOMYCIN-RESISTANCE GENES	ANTIBIOTIC-RESISTANCE PATTERN
			ERM(B)	*MEF(A/E)*	*ERE(B)*	*BLA (TEM AND SHV)*	*VANA*	
1	Surgical ward	Wound	+	–	+			TE, AM, FEP, E, RD, **CN**, AMC, CIP, LNZ
2	Surgical ward	Wound	–	–	+	–		TE, AM, FEP E, RD, CN
3	Surgical ward	Wound	+	–	+	–	+	TE, AM, FEP, E, RD, CN, CIP, AMC, Va
4	Surgical ward	Wound	+	–	+	–	+	TE, AM, FEP, E, RD, CN CIP AMC Va
5	Surgical ward	Wound	+	–	+	–	+	TE, AM, FEP, E, RD, CN, AMC, CIP, Va, LNZ
6	Surgical ward	Wound	–	–	+	–		TE, AM, FEP, E, RD, CN
7	Surgical ward	Wound	+	–	+	–	+	TE, AM, FEP, E, RD, CN, AMC, CIP, Va, LNZ
8	Surgical ward	Wound	–	–	+	–		TE, AM, FEP, E, RD, CN, Va
9	Surgical ward	Wound	+	–	+	–	+	TE, AM, FEP, E, RD, CN, AMC, CIP, Va, IPM, LNZ
10	Surgical ward	Wound	+	–	+	–	+	TE, AM, FEP, E, RD, CN, AMC, CIP, Va
11	Surgical ward	Wound	+	–	+	–	+	TE, AM, FEP, E, RD, CN, AMC, CIP, Va
12	Surgical ward	Wound	–	–	–	–		TE, AM, FEP, E
13	Surgical ward	Wound	+	–	+	–	+	TE, AM, FEP, E, RD, CN AMC, CIP, Va, LNZ
14	Surgical ward	Wound	+	–	+	–	–	TE, AM, FEP, E, RD, CN, AMC, CIP, Va
15	Surgical ward	Wound	+	–	+	–		TE, AM, FEP, E, RD, CN, AMC, CIP
16	Surgical ward	Wound	–	–	–	–		TE, AM, FEP, E, RD,
17	Surgical ward	Wound	–	–	+	–		TE, AM, FEP, E, RD, CN, AMC
18	Surgical ward	Wound	+	–	+	–		TE, AM, FEP, E, RD, CN, CIP
19	Surgical ward	Wound	+	–	–	–		TE, AM, FEP, E, RD, CN, AMC
20	Surgical ward	Wound	+	–	+	–		TE, AM, FEP, E, RD, CN, AMC
21	Surgical ward	Wound	+	–	+	–	+	TE, AM, FEP, E, RD, CN, AMC, CIP, Va, LNZ
22	Surgical ward	Wound	–	–	+	–	–	TE, AM, FEP, E, RD, CN, AMC, Va
23	Surgical ward	Wound	+	–	+	–	+	TE, AM, FEP, E, RD, CN, AMC, CIP, Va, IPM
24	Surgical ward	Wound	+	–	–	–	–	TE, AM, FEP, E, AMC, CIP, Va
25	Surgical ward	Blood	–	–	–	–		TE, AM, FEP, E
26	Surgical ward	Blood	–	–	–	–		TE, AM, FEP

Abbreviations: AM, ampicillin; AMC, amoxicillin-clavulanic; CIP, ciprofloxacin; CN, gentamicin; E, erythromycin; FEP, cefepime; IPM, imipenem; LNZ, linezolid; RD, rifampin; TE, teicoplanin; TE3, tetracycline; Va, vancomycin.

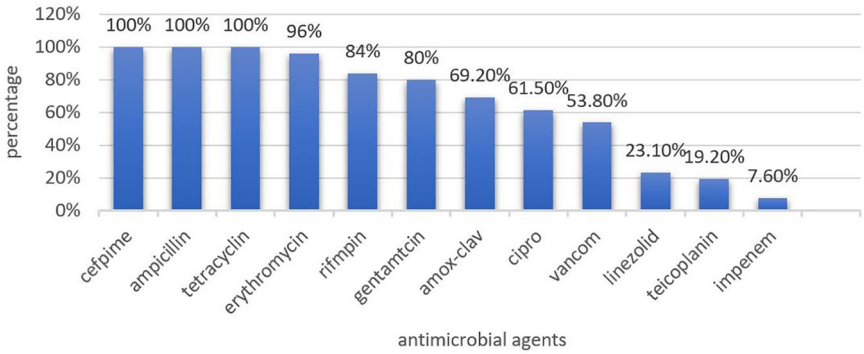

Figure 1. Antimicrobial resistance patterns of *E facalis* isolated from hospital-acquired infections.
Amox-clav indicates amoxacillin-clavulanic acid; cipro, ciprofloxacin, vancom, vancomycin.

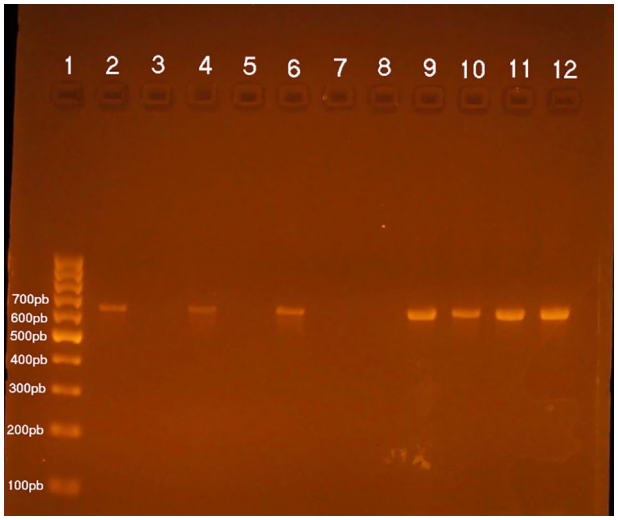

Figure 2. Gel electrophoresis for PCR products detecting erm B gene; lane 1: 100 bp molecular weight marker, lane 2: positive control, lane 3: negative control, lane 4, 6, and lanes 9-12: positive strains (639 bp), lanes 5, 7, and 8: negative strains.

(61.5) of *E faecalis* isolates carried *erm(B)* gene and no isolate was found to harbor the *mef(A/E)* gene. Reyes et al[43] reported that all *enterococcal* isolates with high-level resistance to erythromycin carried the *erm(B)* gene and no isolate was found to harbor the *mef(A/E)* gene. A previous study investigated *erm(B)* and *mef(A/E)* genes in *E Faecalis* isolates from urine samples in our locality reported that, 92.5% (37/40) and 2.5% (1/40) of isolates were positive for *erm(B)* and *mef(A/E)*, respectively.[27] Ribeiro et al[44] reported that 9/20 (45%) of *E Faecalis* isolates carried *ere(B)* gene[41]; the data about *ere(B)* gene is little so our finding may give an important information about the role of *ere(B)* gene in macrolide resistance or cross-resistance not in *E faecalis* only but also in all gram-positive pathogens. *Van A* gene was detected in 71.4% of vancomycin-resistant isolates that was comparable with previous studies [17] and not comparable with others.[36] However, some reports identified *vanA* gene in all vancomycin-resistant isolates.[32] *bla*TEM and blaSHV genes were not detected in our study, which agreed with some of previous studies,[45] and disagreed with others.[24]

In summary, this study showed a prevalence of 8.6% *E faecalis* among 300 Egyptian patients with hospital-acquired infections. MDR in all isolates (100%) and high rates of resistance to gentamicin, erythromycin, and vancomycin were reported in *E faecalis* strain isolated from surgical wound samples and blood cultures, which considers an important health problem in the region. Erythromycin resistance in the studied isolates mainly related to the presence of *ere(B)* and *erm(B)* genes and vancomycin resistance is mainly related to *van A* gene.

In conclusion, occurrence of cross-resistance to macrolides between gram-positive pathogens due to increasing rate of mutations in resistance genes and gene transfer between different species make the studying of such mechanisms between the *enterococcus* isolates still important. Vancomycin resistance studying is also important due to increasing rates all over the world. Linezolid, teicoplanin, and imipenem represent alternative choices for Egyptian patients with hospital-acquired *E faecalis*. Screening studies like this could help in identifying effective treatment measures to control such infections.

Limitations

There are two major limitations in this study that could be addressed in future research: first is that the study focused on *E faecalis* only, and second is the small sample size.

Author Contributions

All authors conducted the the research, wrote the manuscript and analyzed the results. All authors reviewed the final manuscript.

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